

## ***In vitro* investigations of *Cynara scolymus* L. extract on cell physiology of HepG2 liver cells**

**Gesine Löhr, Alexandra Deters, Andreas Hensel\***

*Institute for Pharmaceutical Biology, University of Münster, Germany*

The objective of this study was the investigation of a potential influence of artichoke leaf extract (ALE) on the cell physiology and gene expression of phase I/II enzymes of human liver cells HepG2 and investigation on potential cell protective effects against ethanol-induced cell toxicity against HepG2 cells. Cell biological assays under *in vitro* conditions using HepG2 liver cells and investigation of mitochondrial activity (MTT test), proliferation assay (BrdU incorporation ELISA), LDH as toxicity marker, gene expression analysis by RT-PCR and enzyme activity of glutathione transferase. Artichoke extract, containing 27% caffeoylquinic acids and 7% flavonoids induced mitochondrial activity, proliferation and total protein content under *in vitro* conditions in human liver cells HepG2. These effects could not be correlated to the well-known artichoke secondary compounds cynarin, caffeic acid, chlorogenic acid, luteolin and luteolin-7-*O*-glucoside. The flavones luteolin and luteolin-7-*O*-glucoside had inhibitory effects at 100 µg/mL level on HepG2 cells, with luteolin being a significant stronger inhibitor compared to the respective glucoside. Artichoke leaf extract had minor stimulating effect on gene expression of CYP1A2, while CYP3A4, GGT, GPX2, GSR and GST were slightly inhibited. GST inhibition under *in vitro* conditions was also shown by quantification of GST enzyme activity. Induction of gene expression of CYP1A2 was shown to be supraadditive after simultaneous application of ethanol plus artichoke extract. Artichoke leaf extract exhibited cell protective effects against ethanol-induced toxicity within cotreatment under *in vitro* conditions. Also H<sub>2</sub>O<sub>2</sub> damage was significantly inhibited by simultaneous artichoke incubation. Pre- and posttreatments did not exert protective effects. DMSO-induced toxicity was significantly reduced by pre-, post- and cotreatment with artichoke extract and especially with luteolin-7-*O*-glucoside, indicating a direct interaction with the toxifying agent and an induction of repair mechanisms.

**Uniterms:** *Cynara scolymus* L. Asteraceae. Artichoke. Liver cells. Cell proliferation. Gene expression.

O objetivo deste estudo foi a investigação de uma potente influência do extrato das folhas da alcachofra (ALE) na fisiologia celular e na expressão gênica de enzimas de fase I/II de células hepáticas humanas HepG2 e investigação no potencial efeito protetor celular em células HepG2 contra toxicidade celular induzida por etanol. Ensaios biológicos de células em condições *in vitro* usando células de fígado HepG2 e investigação da atividade mitocondrial (teste MTT), ensaio de proliferação, LDH como marcador de toxicidade, análise de expressão gênica por RT-PCR e atividade da enzima glutathione transferase. O extrato da alcachofra, contendo 27% de ácidos cafeoilquínico e 7% de flavonóides, induzem a atividade mitocondrial, proliferação e o teor de proteína total em condições *in vitro* em células hepáticas humanas HepG2. Estes efeitos não podem ser correlacionados aos compostos secundários conhecidos da alcachofra, cinarina, ácido cafeico, ácido clorogênico, luteolina e luteolin-7-*O*-glicosídeo. As flavonas luteolina e luteolin-7-*O*-glicosídeo possuem efeitos inibitórios em nível de 100 µg/mL em células HepG2, com a luteonina sendo uma inibidora significativamente mais forte comparada com o respectivo glicosídeo. O extrato das folhas de alcachofra possui um efeito mínimo da estimulação na expressão gênica de CYP1A2, enquanto CYP3A4, GGT, GPX2, GSR e GST foram sutilmente inibidos. A inibição de GST em condições *in vitro* também foi mostrada pela quantificação da atividade da enzima GST. Indução da expressão gênica de CYP1A2 mostrou-se supraaditiva após aplicação simultânea do etanol mais o extrato de alcachofra. O extrato das folhas de alcachofra exibiu efeitos protetores celulares frente à toxicidade induzida por etanol em co-tratamento em condições *in vitro*. Além disso, danos por H<sub>2</sub>O<sub>2</sub> foram significativamente inibidos

\*Correspondence: A. Hensel, University of Münster, Institute for Pharmaceutical Biology, Hittorfstr. 56, D-48149 Münster, Germany. e-mail: [ahensel@uni-muenster.de](mailto:ahensel@uni-muenster.de)

pela incubação simultânea do extrato de alcachofra. Pré e pós-tratamento não exerceram efeitos protetores. Toxicidade induzida por DMSO foi significativamente reduzida por pré, pós e co-tratamento com extrato de alcachofra e especialmente com luteína-7-*O*-glicosídeo, indicando uma interação direta com o agente tóxico e a indução dos mecanismos de reparo.

**Unitermos:** *Cynara scolymus* L. Asteraceae. Alcachofra. Células hepáticas. Proliferação celular. Expressão gênica.

## INTRODUCTION

*Cynara scolymus* L. is an herbal medicinal product widely used in functional foods and phytotherapeutics for digestive complaints, adjuvant treatment of moderate hyperlipidaemia and hepato-biliary disorders in traditional European medicine. For review of established clinical use see (ESCOP Monograph, 2003). Traditionally, hepatostimulating effects are described; for review see (Brand, 1992).

The herbal material is phytochemically characterized by caffeoylquinic acids, e.g. chlorogenic acid and 1,5-dicaffeoylquinic acid (cynarin), flavonoids (e.g. luteolin-7-*O*-glucoside and 7-*O*-rutinoside) and bitter tasting sesquiterpene lactones (e.g. cynaropicrin). Various aliphatic acids and caffeic acid derivatives are described.

Concerning the mode of action for treatment of hypercholesterinaemia (Brand, 1990) the reduction of elevated serum cholesterol levels by artichoke is due to an inhibition of incorporation of acetate into the non-saponifiable lipid fraction and thus will reduce cholesterol biosynthesis (Gebhard, 1995; Gebhard, 1996). As assessed by a recent Cochrane review [5] the clinical effects published unto now indicate a clear tendency towards lower serum cholesterol during artichoke therapy. Other benefits as lipid-lowering efficacy are reported, the evidence however is not compelling.

Other traditional uses of artichoke leaf extract are digestive complaints and hepatobiliary disturbances (for review see ESCOP Monograph, 2003). These indications are due to an increased secretion of biliary substances into bile canals (Matuschowski *et al.* 1997; Gebhard, 2001), leading to a choleric effect (Matuschowski *et al.*, 2005). Clinical data on the use of artichoke on this topic are promising and are reviewed in the above mentioned ESCOP monograph. Other traditional uses of artichoke leaf extract are hepatoprotective (Adzet *et al.*, 1987; Preziosi, 1962; Wojcicki, 1978; Gebhard 1997) and liver regenerating effects (Maros *et al.*, 1968).

Because literature does not answer the question on the cellular mode of action concerning hepatotoxicity the aim of the following study was the *in vitro* investigation of artichoke extracts and isolated compounds on cell

physiology of human liver cells and to evaluate potential hepatoprotective effects against different exogenous noxes on physiological and gene expression level.

## MATERIALS AND METHODS

### General

Standardized artichoke extract was obtained from Casellamed, Köln, Germany and was derived from a commercialized extract use for Hepar®SL forte by extraction with aliphatic alcohols (Wittmer *et al.*, 2005). This extract contained 27.5% caffeoylquinic acids with 10.3% specified as 3-caffeoylquinic acids. The sum of flavonoids in the extract was 7.1% with 5.8% of luteolin-7-*O*-glucoside. Cynarin, chlorogenic acid, caffeic acid, luteolin and luteolin-7-*O*-glucoside were obtained by Casellamed AG with purity > 95%. If not stated otherwise, biological assays were performed in 3 independent assays in replicates.

### Testing on human liver cell line

HepG2 (ATCCC HB-8065) cell line, clone H20, capable to express phase I and II metabolizing enzymes under standard cell culture conditions, was obtained from Prof. Mersch-Sundermann, University of Giessen, Germany, and was cultured as described previously (Dauer *et al.*, 2003; Hofmann *et al.*, 2007). Cells were routinely grown in low glucose (1 g/L) Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine and 25 mM Hepes (PAA, Cölbe, Germany) supplemented with 15 % (v/v) heat-inactivated fetal calf serum and gentamycin (30 µg/mL) in a humidified atmosphere at 37±0.5°C, 5% CO<sub>2</sub> (Incubator Binder AG, Germany). The medium was changed every 3-5 days. Test-substances were dissolved at 1 mg/mL in HepG2-medium (if necessary under addition of DMSO as cosolvent, respective control solutions in the same way) and filtered through 0.2 µm cellulose-acetate (Iwaki Glass, Tokyo, Japan).

### Cell assays

Incubation with test compounds occurred over 48 hours: cells were seeded into 96-well cell plates (Greiner, Frickenhausen, Germany) at 1 x 10<sup>4</sup> cells/well. After

24 h, the medium was removed and cells were exposed to 100 and 10  $\mu\text{g/mL}$  of test compounds for 48 h. Cell viability was quantified using the MTT-test according to Mosmann, 1983). Cell proliferation was quantified by BrdU-Incorporation ELISA [18]. Extracytosolic LDH (Porstmann *et al.*, 1985) was quantified by cytotoxicity assay (Roche Diagnostics, Mannheim, Germany). For gene expression analysis, cells were incubated together with test compounds for 24 hours. Total RNA was isolated with Perfect RNA Eukaryotic Mini Kit (Eppendorf, Hamburg) according to the instructions of the manufacturer. The amount of total RNA was estimated by optical density at 260 nm. Equal amounts of RNA were used for reverse transcriptase polymerase chain reaction (RT-PCR) done with TaqMan® Reverse Transcription Reagents® (Applied Biosystems, Darmstadt). Real-Time-PCR was carried out using the TaqMan® Universal PCR Master Mix and specific TaqMan® gene expression assays (from Applied Biosystems, Darmstadt, Germany):

genes	Assay ID
GPX	Hs00702173_s1
CYP1A2	Hs00167927_m1
CYP3A4	Hs00604506_m1
GGT1	Hs00359124_g1
GSR	Hs00167317_m1
GST	Hs00542846_m1
GAPDH	Hs99999905_m1

For GST no distinct isoforms were screened but the protein coding gene type GST SCLo6A1 from the HGNC 23613 primary source with a validated RefSeq status from human gene identification number Hs.388874 from chromosome No. 5 Matuschowsky *et al.*, 1997). Glyceraldehyd-3-phosphate dehydrogenase was used as the internal reference. Data were quantified by the 7300 Real-time PCR System RQ Study software (Applied Biosystems, Darmstadt). Gene expression was monitored in 3 independent assays.

### Protein biochemistry

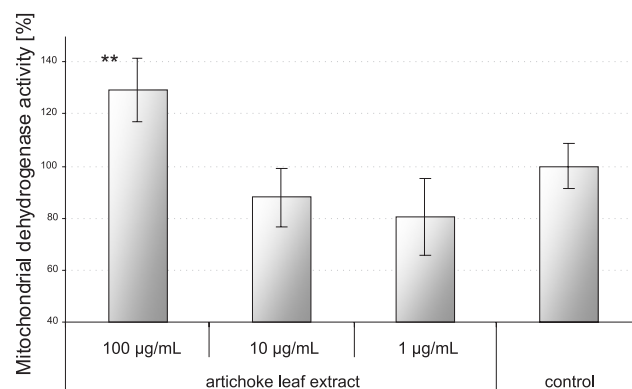
**Protein isolation:** Adherent cells were washed two times with ice cold water, followed by treatment with lyses buffer (NaPP buffer, 50 mM, pH 6.8, 5% SDS, 40 mM DTT, 5mM EDTA, 5 mM EGTA, 15% glycerine, 0.1% Triton®X-100). Cells were harvested, transferred into a tube and lysed by sonication for 3 x 10 sec. Supernatant was used after centrifugation (12.000xg) for protein determination according to (Lowry *et al.*, 1951). GST was

determined using the GST determination of Novagen (San Diego, U.S.A) (Suzuki *et al.*, 2003).

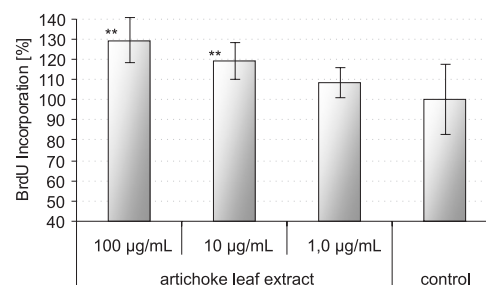
## RESULTS

An artichoke leaf extract (ALE) containing 27% caffeoylquinic acids and 7% flavonoids (Wittermer *et al.*, 2005) significantly increased the mitochondrial dehydrogenase activities of human liver HepG2 after 48 h incubation time at a 100  $\mu\text{g/mL}$  level, while lower doses were ineffective (Fig. 1A). This was confirmed by the results of proliferation test: ALE significantly increased proliferation rate at doses of 100  $\mu\text{g/mL}$  to about 130% proliferation, and at 10  $\mu\text{g/mL}$  to 120%; lower doses were ineffective (Fig. 1B). Additionally a significant increase in protein content in treated HepG2 cells was measured: ALE at 100  $\mu\text{g/mL}$  significantly induced the protein content of the cells to about 130% (data not shown), reflecting the increase in cell number and energy turn over.

A



B



**FIGURE 1** - Influence of artichoke leaf extract (ALE) at 1, 10 and 100  $\mu\text{g/mL}$  level on mitochondrial dehydrogenase activity (A) and mitotic cell proliferation (B) of HepG2 cells after 48 hours incubation. Mitochondrial activity determined by MTT test, proliferation determination by BrdU incorporation ELISA. The bars represent standard errors SD from 3 independent experiments with  $n = 10$  replicates each with  $*p < 0.05$ ,  $**p < 0.01$  compared to the untreated control group.

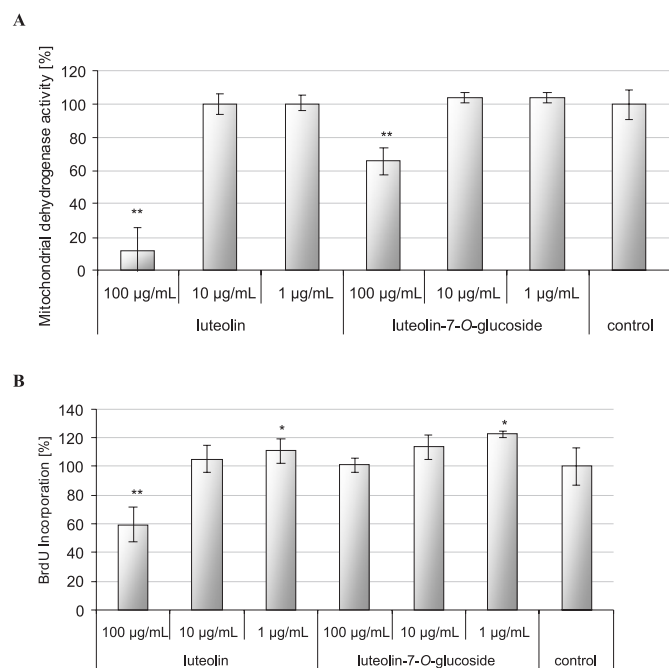
No necrotic and apoptotic cytotoxicity of ALE was observed within the test range of 1 to 100 µg/mL as measured by LDH determination and inspection on apoptotic bodies.

To investigate the influence of isolated artichoke compounds on HepG2 cells reference compounds luteolin, luteolin-7-*O*-glucosid, caffeic acid, chlorogenic acid and cynarin were tested at 100, 10 and 1 µg/mL level. Within MTT assay cynarin, chlorogenic acid and caffeic acid did not exhibit any significant effect. Luteolin and luteolin-7-*O*-glucosid at 100 µg/mL ( $3.5 \times 10^{-7}$  resp.  $2.3 \times 10^{-7}$  mol/L) significantly decreased mitochondrial activity (Fig. 2A), related to the calculation of absolute amounts of 100 µg/mL of both compounds. Also when the measured data were normalized to equimolar concentrations ( $3.5 \times 10^{-7}$  mol/L Luteolin with 84 % inhibition,  $3.5 \times 10^{-7}$  mol/L, luteolin-7-*O*-glucoside with 54 % inhibition) significantly higher inhibition of mitochondrial activity was obvious for luteolin compared to the respective glycoside. This was also reflected within the respective proliferation assays (Fig. 2B). The inhibitory effect of the flavone-glucosid was significantly lower than that of the free aglycon luteolin.

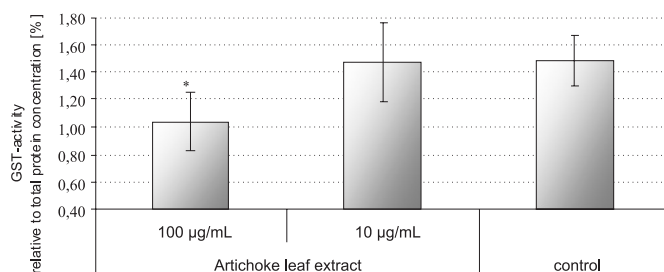
The mRNA expression of different phase I and II enzymes of HepG2 cells was determined by quantitative RT-

PCR after a 24 h incubation time of cells with and without ALE in serum-free media. Gene expression was monitored in 3 independent assays for glutathione-S-transferase GST, glutathionperoxidase GPX, glutathionreductase GSR,  $\gamma$ -glutamyltransferase GGT1 and the cytochrome P450 isoforms CYP1A2 and CYP3A4. These CYPs were selected because CYP3A4 is the main hepatic cytochrom (about 30%) beside CYP1A2 (about 10 to 12%). Measured test values were related to the expression of the endogenous control glycerol-3-phosphate dehydrogenase (GAPDH). mRNA expression for CYP1A2 was slightly increased in the presence of artichoke extract ALE (100 µg/mL) to  $1.76 \pm 2.4$ . Because of the high variability this effect is assessed not to be significant. Significant inhibition of gene expression by ALE was seen for CYP3A4 ( $0.23 \pm 0.39$ ), for GGT1 ( $0.59 \pm 0.21$ ), for GPX2 ( $0.50 \pm 0.30$ ), for GSR ( $0.72 \pm 0.23$ ) and GST ( $0.22 \pm 0.28$ ).

The inhibition of glutathione-S-transferase GST gene expression was confirmed by determination of the respective enzyme activity. Therefore HepG2 cells were incubated with ALE (100, 10 µg/mL) for 48 h. Total protein was isolated from cell lysate and GST activity was determined after conjugation of 1-chloro-2,4-dinitrobenzene CDNB to endogenous GST. GST activity was calculated relative to the total cell protein content against a GST calibration curve in ALE-treated cells and untreated control. As shown in Fig. 3 ALE inhibited GST activity in a dose-dependent manner.



**FIGURE 2** - Influence of luteolin and luteolin-7-*O*-glucoside at 1, 10, 100 µg/mL on mitochondrial dehydrogenase activity (A) and mitotic cell proliferation (B) of HepG2 cells after 48 hours of incubation. Mitochondrial activity was determined by MTT test, proliferation was determined by BrdU incorporation ELISA. The bars represent SD from independent assay with  $n = 10$  replicates with  $*p < 0.05$ ,  $**p < 0.01$  compared to the untreated control group.



**FIGURE 3** - Concentration-dependent inhibition of glutathione-S-transferase (GST) in HepG2 cells after 48 h treatment with ALE relative to the total protein concentration. The bars represent SD with  $n = 10$  replicates  $*p < 0.05$ .

Summarizing at this point, artichoke extract is assessed to have stimulating activity on the proliferation and the energy turn-over of liver cells, accompanied by effects on metabolising enzymes.

In order to investigate if artichoke extract has also anti-toxic effects against exogenous chemicals, ALE was tested as cytoprotective activity against the toxifying effects of ethanol (marker for unspecific enzyme toxicity), hydrogenperoxide (oxidative toxicity) and dimethylsulfo-



xid (necrotic toxicity). In a preliminary study the respective concentrations and incubation times were verified to induce a cell toxicity, equivalent to about 50% reduction of MTT activity. Ethanol was used in the following studies at a 25 to 50 mM level,  $H_2O_2$  at 1 to 10 mM, and DMSO at 3.5 and 5% concentration over a 6 to 24 hours incubation time.

As shown in Table I, cotreatment of HepG2 cells with ALE (100  $\mu$ g/mL) and ethanol (25 and 50 mM) for 48 hours showed a significant protective effect. Also at 10  $\mu$ g level the respective data showed (not significant) tendencies towards cytoprotective effects.

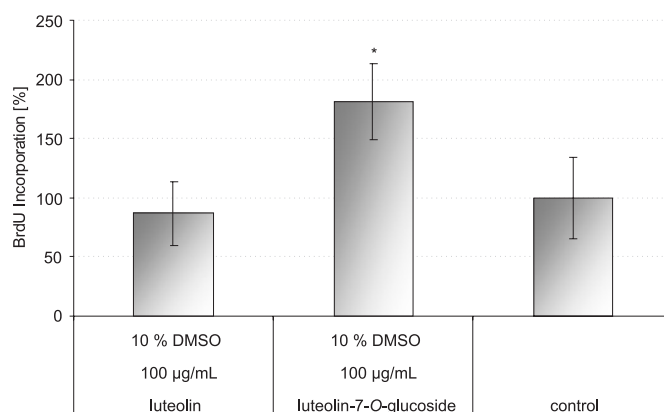
Also cotreatment of HepG2 cells with ALE (100  $\mu$ g/mL) and  $H_2O_2$  (1, 5, 10 mM) for 24 hours showed a significant protective effect against the low-dose hydrogen peroxide induced toxicity: while only minor antioxidative effects of ALE were seen against strong cell damage induced by 10 mM  $H_2O_2$ , clear effects of ALE were observed against 5 mM  $H_2O_2$ .

Coincubation of HepG2 cells with DMSO (3.5 and 5%) and ALE for 48 hours (10, 100  $\mu$ g/mL) indicated reduced DMSO-induced toxicity at the 100  $\mu$ g/mL level, while lower doses did not protect from DMSO-mediated cell toxicity.

Similar test systems, but with pre- and postincubation of cells with ethanol,  $H_2O_2$  or DMSO prior or after an ALE-treatment, did not reduce the toxic effects of the chemicals towards HepG2 cells.

In addition to these experiments with artichoke extract, a potential cytoprotective effect of the flavonole reference compounds luteolin and luteolin-7-*O*-glucoside was investigated against DMSO as exogenous stress fac-

tor. For that purpose one cell group, serving as a control, was treated with DMSO 10%. Proliferation behaviour of this group - with a significantly reduced vitality in contrast to an untreated control - was calculated as 100%. Coincubation of two cell groups with luteolin + DMSO 10% resp. luteolin-7-*O*-glucoside + DMSO 10% indicated that the flavone-glycoside significantly protects cells against DMSO-related toxicity (Fig. 4).



**FIGURE 4** - Proliferation (BrdU-ELISA) of HepG2 cells within 48 h cotreatment with DMSO 10% and 100  $\mu$ g/mL luteolin (0.35  $\mu$ M/mL) resp. luteolin-7-*O*-glucoside (0.22  $\mu$ M/mL). \* $p < 0.05$  compared to the DMSO 10% control group.

For investigation of the cytoprotective effects of ALE against ethanol-induced cell damage the mRNA expression of different phase I and II liver enzymes was determined after 3 different treatment cycles of HepG2 cells over a 24 h incubation time (pre-, post- and cotreatment

**TABLE I** - Influence of different pre-, post- and cotreatment regimes of ethanol, hydrogen peroxide and DMSO in combination with artichoke extract (ALE) on mitochondrial activity of HepG2 cells. Mitochondrial activity was determined by MTT test and measured values were related to the respective control groups (100%). \*\* $p < 0.01$ .  $\Rightarrow$ : followed by treatment with compounds indicated

Type of treatment of HepG2 cells			Mitochondrial Activity (%)
Ethanol 50 mM; 48 h	$\Rightarrow$	ALE 100 $\mu$ g; 48 h	313 +/- 38% **
Ethanol 25 mM; 48 h	$\Rightarrow$	ALE 100 $\mu$ g; 48 h	178 +/- 77% **
Ethanol 50 mM; 48 h	$\Rightarrow$	ALE 10 $\mu$ g; 48 h	283 +/- 111%
Ethanol 25mM; 48 h	$\Rightarrow$	ALE 10 $\mu$ g; 48 h	168 +/- 60%
$H_2O_2$ 10 mM; 24 h	$\Rightarrow$	ALE 100 $\mu$ g; 24 h	116 +/- 2%
$H_2O_2$ 5 mM; 24 h	$\Rightarrow$	ALE 100 $\mu$ g; 24 h	263 +/- 92% **
$H_2O_2$ 1 mM; 24 h	$\Rightarrow$	ALE 100 $\mu$ g; 24 h	107 +/- 12%
DMSO 5%; 48 h	$\Rightarrow$	ALE 100 $\mu$ g; 48 h	100 +/- 12 %
DMSO 5%; 48 h	$\Rightarrow$	ALE 10 $\mu$ g; 48 h	97 +/- 7 %
DMSO 3.5%; 48 h	$\Rightarrow$	ALE 100 $\mu$ g; 48 h	137 +/- 6 % **
DMSO 3.5%; 48 h	$\Rightarrow$	ALE 10 $\mu$ g; 48 h	101 +/- 4 %

**TABLE II** - Influence of artichoke extract (ALE, 100 µg/mL) in pre-, post- and cotreatment experiments with ethanol (50 mM) on relative gene expression on cytochromes CYP1A2 and CYP3A4 by quantitative real time PCR (TaqMan® assay), normalized to the house keeping gene GAPDH and untreated controls. ⇒: followed by treatment with compounds indicated

Treatment of HepG2 cells			CYP1A2	CYP3A4
Ethanol 50 mM; 1 h	⇒	ALE 100 µg; 23 h	3.3	1.0
Control experiment: Ethanol 50 mM; 1 h	⇒	Medium only; 23 h	3.0	2.5
ALE 100 µg; 23 h	⇒	Ethanol 50 mM; 1 h	7.1	1.7
Control experiment: Medium only; 23 h	⇒	Ethanol 50 mM; 1 h	6.2	1.0
Ethanol 50 mM; 24 h	+	ALE 100 µg; 24 h	29.8	3.9
Control experiment: Medium	+	ALE 100 µg; 24 h	1.8	0.7

of cells with ethanol 50 mM and ALE 100 µg/mL). Gene expression was monitored for GST, GPX, GSR, GGT1, CYP1A2 and CYP3A4 and all values were related to the expression of the endogenous control GAPDH. As shown in Table II ethanol 50 mM increased mRNA levels of CYP1A2 significantly. Pre- and posttreatment experiments with ALE revealed no big differences between the different test groups. In contrast to that, a thirtyfold increase in CYP1A2 was observed when coincubation was performed with ethanol plus ALE for a 24 h incubation period, indicating a strong influence of the artichoke-ethanol mixture on metabolic activity of the liver cells.

Significant influences of ethanol and/or ALE on expression of GST, GPX, GSR, GGT1 were not observed.

## CONCLUSIONS

Extracts of artichoke are widely used for its choleretic and antihypercholesterinaemic effects. Within literature potential hepatoprotective effects are described, mainly using *in vivo* rat models (Gebhard, 1997). *In vitro* studies of hepatoprotective effects of artichoke extracts on cultured rat hepatocytes are shown against t-butylhydroperoxide-induced peroxidation of membrane lipids (Gebhard, 1997). Additionally cyanarin and caffeic acid were shown to reduce the carbontetrachloride-induced leakage of liver enzymes glutamic oxaloacetic transaminase and glutamic pyruvic transaminase (Adzet *et al.*, 1987).

These data correlate with our investigations, indicating artichoke extract to have a direct effect on liver cells. Cell physiology of HepG2 cells is clearly up-regulated, with increased mitochondrial activity, leading to a higher energy status, inducing an increased protein synthesis and ending in a higher mitogenic activity. On the other side it is absolutely unclear which compounds from artichoke are responsible for these effects. In our investigations neither cyanarin, chlorogenic acid or caffeic acid had any influence on the energy status of the cells. From that point

of view artichoke extract specifications for preparations marketed with the indication "hepatoprotection" should not be standardized on these compounds. On the other side the flavones were shown to have strong inhibitory effects on liver cells with luteolin being a quite strong inducer of apoptosis by inhibition of topoisomerase II (Cantero *et al.*, 2006). It seems interesting that luteolin-7-*O*-glucoside has significantly less toxic effects on HepG2 cells rather than the free aglycon luteolin, probably by a reduced absorption into the cells. For manufacture of commercial artichoke leaf extracts specifications therefore should focus on the amount of luteolin-glucoside beside that of the free aglycon.

Because the protein content of the cells is increased during incubation with artichoke extract it could be argued that also the metabolizing enzymes are upregulated unspecifically. This is clearly not the case when looking at the gene expression data of phase I and II enzymes expressed in HepG2 cells. Only one cytochrome mRNA, namely CYP1A2 is upregulated to a minor degree which is assessed to have no clinical relevance. All other enzymes monitored were slightly inhibited. This was also confirmed by biochemical determination of enzyme activity of GST which was significantly down-regulated by artichoke extract. If this enzyme inhibition has any clinical relevance this has to be investigated by *in vivo* studies in future.

Beside the direct effect of artichoke extract on HepG2 cells, pronounced cell protection by ALE was obvious. This was not only due to the known antioxidative effects of artichoke which were obvious in our studies against hydrogen peroxide and in previous studies (Gebhard, 1997) against t-BHP. Cell protection of ALE can also be discussed on ethanol-induced cell damage over metabolic pathways as well as against necrotic membrane disturbance, induced by compounds as DMSO. In general direct interactions between toxifying agents and ALE can be claimed as shown by the respective cotreatment experiments. On the other side also indirect effects are induced

by artichoke extracts. The posttreatment experiments with ALE after damage with DMSO clearly confirmed the induction of repair mechanisms. A prophylactic application of artichoke extract does not seem to have an effect. On the other side it has to be pointed out that the positive effects of ALE against EtOH-, H<sub>2</sub>O<sub>2</sub>- and DMSO-damaged liver cells is not due to a detoxificant effect but more due to an stimulation of metabolic capacity of the cells. Summarizing artichoke leaf extract may have the potential to be used as hepatoprotective agent. Prospective research will identify those compounds, which are responsible for described effects.

## ACKNOWLEDGEMENTS

The HepG2 cell line was a generous gift from Prof. Dr. Mersch-Sundermann is acknowledged. Financial support by Lichtwer AG / Casellamed AG is acknowledged.

## REFERENCES

- ADZET, T.; CAMARASA, J.; LAGUNA, J. Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl<sub>4</sub> toxicity in isolated rat hepatocytes. *J. Nat. Prod.*, v.50, p.612-617, 1987.
- BRAND, N.; *Cynara*. In: HÄNSEL, R.; SÉLLER, K., RIMPLER, H., SCHNEIDER, G., (Eds.). *Hagers Handbuch der Pharmazeutischen Praxis, Drogen*. 5. ed. Berlin, Heidelberg, New York: Springer Publishers, 1992. v.4, p.1117-1131.
- CANTERO, G.; CAMPANELLA, C.; MATEOS, S.; CORTES, F. Topoisomerase II inhibition and high yield of endoreduplication induced by the flavonoids luteolin and quercetin. *Mutagenesis*, v.21, p.321-325, 2006.
- DAUER, A.; HENSEL, A.; LHOSTE, F.; KNASMUELLER, S.; MERSCH-SUNDERMANN, V. Genotoxic and antigenotoxic effects of catechin and tannins from the bark of *Hamamelis virginiana* L. in metabolically competent, human hepatoma cells (HepG2) using single cell electrophoresis. *Phytochem.*, v.63, p.199-207, 2003.
- ESCOP Monograph – *The Scientific Foundation for Herbal Medicinal Products*. *Cynarae folium - Artichoke leaf*. 2.ed. New York: Thieme Publishers, 2003.
- GEBHARD, R. Anticholestatic activity of flavonoids from artichoke (*Cynara scolymus* L.) and their metabolites. *Med. Sci. Mon.*, v.7, suppl.1, p.316-320, 2001.
- GEBHARD, R. Antioxidative and protective properties of extracts from leaves of the artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Tox. Apl. Pharmacol.*, v.144, p.279-286, 1997.
- GEBHARD, R. Artischockenextrakt – *in vitro* Nachweis einer Hemmwirkung auf die Cholesterolsynthese. *Medizin Welt*, v.46, p.348-350, 1995.
- GEBHARD, R. Neue Erkenntnisse zur Wirkung von Artischockenextrakte. *Z. Allgemeinmed.*, v.72, p.20-23, 1996.
- HOFMANN, T.; DETERS, A.; MÜLLER, G.; STARK, T.; WITTSCHIER, N.; HENSEL, A. Occurrence of N-Phenylpropenoyl-L-amino acids in different herbal drugs and influence on human keratinocytes, human liver cells and against adhesion of *H. pylori* to human stomach. *Planta Med.*, v.73, p.142-150, 2007.
- LOWRY, O.H.; NIRA, J.; ROSEBROUGH, N.J.; FARR, A.L.; RANDALL, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* v.193, p.265-275, 1951.
- MAROS, T.; SERES-STRUMR, L.; RAIZ, G.; RETEJI, C.; KOVACS, V.V.; HINTS, M. Wirkung der *Cynara scolymus*-Extrakte auf die Regeneration der Rattenleber *Drug Res.*, v.18, p.884-886, 1968.
- MARTIN, A.; CLYNES, M. Comparison of 5 microplate colorimetric assays for *in vitro* cytotoxicity testing and cell proliferation assays. *Cytotechnology*, v.11, p.9-58, 1993.
- MATUSCHOWSKI, P.; GUMBINGER, H.G.; NAHRSTEDT, A.; WINTERHOFF, H. Testing of *Cynara scolymus* L. in the isolated perfused rat liver. *Planta Med.*, v. 63, p. 55-56, 1997.,
- MATUSCHOWSKI, P.; NAHRSTEDT, A.; WINTERHOFF, H. Pharmakologische Untersuchungen eines Frischpflanzenpresssaftes aus *Cynara scolymus* auf choleretische Wirkung. *Z. Phytother.*, v.26, p.216-221, 2005.
- MOSMANN, M. Rapid colorimetric assay for cellular growth and survival: applications to Proliferation and cytotoxicity assays. *J. Immunol. Methods*, v.65, p.55-63, 1983.

- PITTLER, M.H.; THOMPSON, C.J.; ERNST, E. Artichoke leaf extract for treating hypercholesterolaemia (Review). *The Cochrane Database of Syst. Rev.*, volume?, Issue 3, pages?, 2002.
- PORSTMANN, T.; TERNYK, T.; AVRAMEAS, S. Quantification of 5-bromo-2'-deoxyuridine into DNA: an enzyme immunoassay for the assessment of the lymphoid cell proliferative response. *J. Immunol. Methods*, v.82, p.169-179, 1985.
- PREZIOSI, P. Dal *Cynara scolymus* allacito 1,4-dicaffeilchinico. *Il Fàrmaco (ed. Sc.)*, v. 17, p. 701-745, 1962.
- STOEV, S.D.; STEFANOV, M.; DENEV, S.; RADIC, B.; DOMINAN, A.M.; PERAICA, M. Experimental mycotoxicosis in Chickens induced by ochratoxin A and penicillic acid and intervention with natural plant extracts. *Vet. Res. Común.*, v.28, p.724-46, 2004.
- SUZUKI, T.; ONOGAWA, T.; ASANDO, N.; MIZUTAMARI, H.; MAKKAICHI, T.; TANEMOTO, M.; *et al.* Identification and characterization of novel rat and human gonad-specific organic anion transporters. *Mol. Endocrinol.*, v.17, p.1203-1215, 2003.
- WITTEMER, S.M.; PLOCH, M.; WINDECK, T.; MÜLLER, S.C.; DREWELow, B.; DERENDORF, H.; *et al.* Bioavailability and pharmacokinetic properties of caffeoylquinic acids and flavonoids after oral administration of artichoke leaf extracts in human. *Phytomedicine*, v.12, p.25-38, 2005.
- WOJCICKI, J. Effect of 1,5-dicaffeoylquinic acid (cynarine) on cholesterol levels in serum and liver of acute ethanol-induced rats. *Drug Alc. Dep.*, v.3, p.143-145, 1978.

Received for publication on 22<sup>th</sup> december 2008

Accepted for publication on 18<sup>th</sup> may 2009